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TECHNICAL MEMORANDUM 225

ABSORPTION, DISTRIBUTION, AND ROOT EXUDATION OF 2,4,5-T AND PICLORAM BY ASH AND MAPLE

Charles P. P. Reid
Woodland Hurtt
William A. Wells

MARCH 1971

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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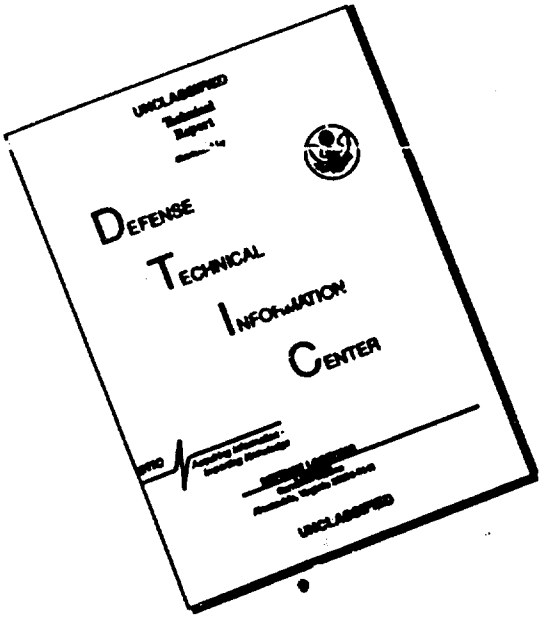

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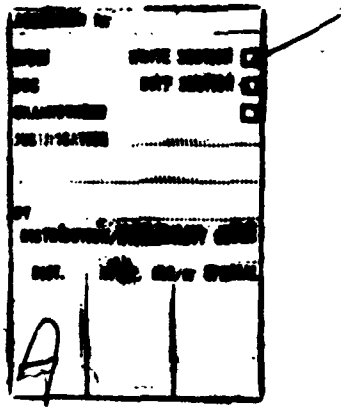

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ABSORPTION, DISTRIBUTION, AND ROOT EXUDATION OF 2,4,5-T AND PICLORAM BY ASH AND MAPLE

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Plant Physiology Division
PLANT SCIENCES LABORATORIES

Project 1B562605AD28

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ABSTRACT

Liquid scintillation and autoradiography were utilized in conjunction with paper chromatography to study the uptake, distribution, and root exudation of foliarly applied C^{14}-picloram (4-amino-3,5,6-trichloropicolinic acid) and C^{14}-2,4,5-T (2,4,5-trichlorophenoxyacetic acid) in red maple, green ash, and white ash.

A leaf-washing technique, which allowed direct counting of the herbicide retained on the surface of the treated leaves, revealed that a greater amount of 2,4,5-T than of picloram was washed off the leaves of all species after 22 days. Greater penetration of picloram was noted when the herbicide was applied with 0.2% Tween 20 as opposed to application with 95% ethanol.

Autoradiography indicated that 2,4,5-T was distributed throughout ash and maple tissues in greater quantities than picloram, and microautoradiography of green ash stem tissue showed possible xylem to phloem exchange. Both picloram and 2,4,5-T were translocated acropetally and basipetally in all species, indicating utilization of both the apoplast and symplast by these two herbicides.

Significant root loss of both picloram and 2,4,5-T was demonstrated in all three species. Greater root loss of both herbicides occurred in red maple than in the two species of ash.

Bioassay in conjunction with paper chromatography and liquid scintillation counting suggested that the C^{14} activity in the exudates was associated with the unaltered herbicide molecules for both 2,4,5-T and picloram. The quantities of 2,4,5-T and picloram exuded from the roots appeared to have little relationship to herbicide tolerance or resistance of these species.

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I. INTRODUCTION

Reports on the use of picloram (4-amino-3,5,6-trichloropicolinic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) for woody plant growth control have indicated that, generally, Fraxinus spp. are resistant to picloram and red maple (Acer rubrum) is susceptible, while both groups of trees are relatively tolerant to 2,4,5-T.[1-5]

The differences in response of ash and maple to picloram and 2,4,5-T have not been completely explained. In an attempt to explain these differences, investigators have examined primarily foliar uptake mechanisms and phloem and xylem transport systems.[1,3,4] Because picloram has been reported to be excreted from roots of bean,[6] it was considered of interest to examine the possibility that tolerance to picloram or 2,4,5-T is related to root excretion. Root excretion has been suggested as a means of detoxification of foliarly applied herbicides.[5] Because there are numerous reports that substances, both endogenous and exogenous, are excreted from root systems by a wide range of plants,[7-10] it was suspected that ash and maple might also excrete the herbicides picloram and 2,4,5-T.

Therefore, the objective of these investigations was to determine if picloram and 2,4,5-T are excreted by roots of ash and maple, and, if so, if they are excreted in sufficient quantities to explain differential tolerance to the two herbicides.

II. METHODS AND MATERIALS

A. RED MAPLE AND GREEN ASH TREATMENTS

Red maple (Acer rubrum) seedlings grown from seed and green ash (Fraxinus pennsylvanica) seedlings obtained from commercial nursery stock were selected for use on the basis of uniformity of size (stem and root lengths of approximately 40 and 25 cm, respectively, and seven to 11 whorls of leaves). Red maple seedlings were 3 months old and green ash seedlings were 20 months old.

Trees were maintained throughout the investigations in a controlled-environment growth chamber at 25±1 C and 45±5% RH by day, 23±1 C and 50±5% RH at night, and a 16-hour photoperiod of 1,900 ft-c at 45 cm above the bench surface. The trees were initially placed in 150-ml aluminum foil - covered test tubes containing 100 ml of aerated 0.5X nutrient solution.[11] Nutrient solution was replenished twice daily. After 10 days, the trees were transferred to foil-covered pint jars containing 300 ml of aerated nutrient solution that was replenished daily. One day after the establishment of the trees in nutrient solution, six trees of each species were treated with previously determined sublethal dosages of C^{14}-labeled picloram (4.25 μc/mg) or with C^{14}-labeled 2,4,5-T (4.25 μc/mg), both labeled at the carboxyl position and prepared in 95% ethanol. Each plant was treated with 0.1 μc of herbicide by applying ten 5-μl droplets to each of two leaves at the fourth whorl above the root collar. The herbicide was applied within lanolin rings on the upper surface of the leaf by a calibrated microliter syringe. Species and herbicide treatment were arranged in a nonrandom alternating pattern on the bench surface.

During the first 7 days after isotope application, 5-ml samples were removed daily from the nutrient solution bathing the roots after the solution in each container was brought up to a predetermined volume. After 7 days, 5-ml samples were removed every 2 to 3 days until end of the experiment. Nutrient solution samples were evaporated under vacuum to dryness, liquid scintillation solution was added, and the samples were counted in a liquid scintillation spectrometer to determine the quantity of C^{14} present. Background determinations were made on nutrient solution of trees receiving no isotope treatment. Twenty-two days after isotope application, the experiment was terminated. Five-milliliter samples were again removed from the nutrient solution after determining the volume of solution bathing the roots of each tree. The activity of this sample was used for determining the total C^{14} lost by the root systems.

The nutrient solutions were combined according to treatment, filtered through Whatman No. 1 paper, and evaporated under vacuum with eight subsequent filtrations through Whatman No. 50 paper until the volume was reduced 435-fold. Ten microliters of the concentrated nutrient solution

from the respective treatments were spotted on Whatman No. 20 paper for descending chromatography with acetone:water (90:10 v/v). Five microliters of C^{14}-labeled picloram or 2,4,5-T were co-chromatographed with the nutrient solution samples as reference standards. After thorough drying in a hood to remove all traces of the solvent, the papers were cut into 1-inch squares, digested, and counted in liquid scintillation for detection of C^{14}. Prior to digesting the 1-inch squares for liquid scintillation counting, the papers containing nutrient solution from the picloram treatments were bioassayed with lettuce seed[12] as an additional means of ascertaining the location of picloram on the chromatograms.

At the end of the experiment, the number of leaf whorls, stem and root length, root volume, and pH of the bathing solution were determined. The treated pair of leaves from each plant was excised and the upper surfaces were rinsed with 14 ml of scintillation solution per leaf directly into scintillation vials. Two-millimeter stem sections above and below the treated leaf whorl and a 2-mm petiole sample were removed from two trees of each treatment and used for histological examination by microautoradiography.

The sections were prepared for microautoradiography by using a slightly modified version of the method of Pickering[13]. The sections were mounted in 5% gelatin (w/v), quick-frozen, and sectioned at 16 μ in a cryostat. The 16-μ sections were attached to microscope slides by a chrome-alum subbing solution (adhesive). Nuclear track emulsion film was applied to each slide in the dark by dipping a wire loop into emulsion maintained at 40 C, allowing the loop of emulsion to partially dry in air for 3 to 5 minutes, and then pressing the film against the tissue. The films were then exposed to the tissue for 19 to 21 days at 8 C in light-tight containers. After exposure, the films were developed for 5 minutes in Kodak D-19 developer at 20 C, fixed in Kodak Photofix for 5 minutes, and rinsed in water for 1 hour. Tissues on the slides were rehydrated and examined as wet mounts.

After surface rinsing, the treated leaves were quick-frozen, lyophilized, and autoradiographed on Kodak No-Screen x-ray film. After they were autoradiographed, each leaf was cut into four sections, placed in vials, digested as reported previously[14], and counted in liquid scintillation.

All trees were lyophilized, pressed and mounted, and autoradiographed[15]. Stem leaf and root samples were removed from two trees of each treatment after the autoradiographs of the whole trees were completed. These tissue samples were digested and counted in liquid scintillation to provide quantitative values for C^{14} for comparison with autoradiographic images.

B. WHITE ASH TREATMENT

White ash (Fraxinus americana) seedlings were grown from seed. When 5 weeks old and 15 to 20 cm from the cotyledonary node to the apex, the seedlings were placed in 1-quart plastic pots (two trees per pot) containing

800 ml of aerated 0.5X nutrient solution. Plants were maintained in a controlled-environment growth chamber at 26±0.5 C and 55±5% RH by day, 23±0.5 C and 65±5% RH at night, and a 16-hour photoperiod of about 1,900 ft-c. Carboxyl-labeled C^{14}-picloram (100 µl) in distilled water with 0.2% Tween 20 (4.25 µc/mg) was applied to each of 16 trees. Two leaves on each plant at the fourth whorl above the cotyledonary node received 0.1 µc as ten 5-µl droplets per leaf. The droplets were applied by microliter pipettes and placed within lanolin rings on the upper surface of the leaf. Four trees not treated were used as a basis for background in radioactive determination.

To determine the loss of C^{14} from the roots to the nutrient solution, 5-ml samples were removed daily after bringing the volume of each container back to 800 ml. The samples were then evaporated under vacuum to dryness and counted in liquid scintillation. Prior to removing the last daily 5-ml sample at the end of the experiment, the volume of nutrient solution in each container was measured. This volume was then used to make the proper adjustment in calculating the total C^{14} recovered in the nutrient solution over the 9-day period.

III. RESULTS AND DISCUSSION

A. RED MAPLE AND GREEN ASH TREATMENTS

The loss of C^{14} to the root bathing solution for 22 days by the ash and maple after treatment with C^{14}-picloram and C^{14}-2,4,5-T is shown in Figure 1. The radioactivity of the nutrient solution represents the mean cumulative total of C^{14} for the replicates at each particular sampling period. The loss of C^{14} from the root system did not appear to differ greatly for the two herbicides within a particular species. However, the loss of C^{14} was greater from maple than ash for both herbicide treatments. The drop in cumulative radioactivity of the solution at 16 days for the maple treated with 2,4,5-T is not easily explained. One possible explanation is a sudden change in recycling of C^{14} from the nutrient solution to the plants.

An attempt was made to correlate the loss of C^{14} from the roots with the volume of the root system at 22 days. No combination of species and herbicide applied was found to have a strong correlation by the method of simple linear correlation[16].

To determine if the C^{14} detected in the nutrient solution was the intact labeled herbicide molecule, the nutrient solutions of the four treatment combinations were examined by paper chromatography. When the nutrient solutions from the ash and maple treated with C^{14}-2,4,5-T were chromatographed, the R_F of the areas of C^{14} activity corresponded closely with the R_F of the reference C^{14}-2,4,5-T spot (Fig. 2). No other radioactive areas were detected on the chromatogram strips.

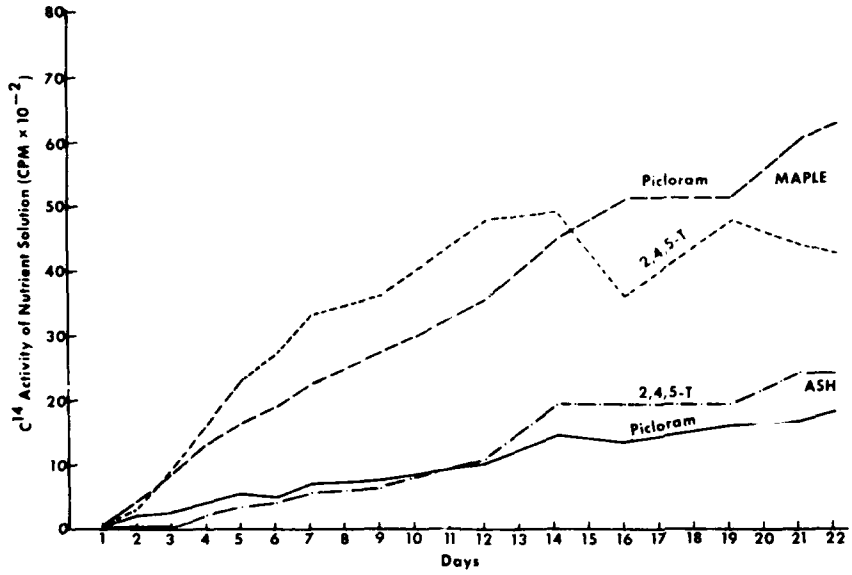

FIGURE 1. Cumulative C^{14} Loss from Roots of Red Maple and Green Ash After Foliar Treatment with 0.1 μc (24 μg) of C^{14}-Picloram and C^{14}-2,4,5-T. Each plotted line represents the mean of six replications.

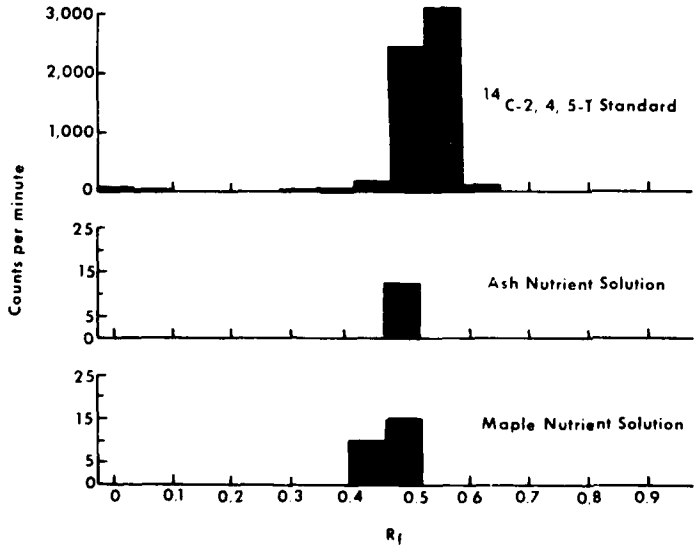

FIGURE 2. Areas of C^{14} Activity on Paper Chromatographs of Nutrient Solutions from Ash and Maple Trees Treated with C^{14}-2,4,5-T and of a C^{14}-2,4,5-T Standard Solution. Values in cpm, corrected for background and counting efficiency, are significantly above background at the 95% confidence level.

The R_F of the C^{14} activity on the chromatograms of the nutrient solutions from the ash and maple treated with picloram also corresponded closely to the R_F of the chromatographed C^{14}-picloram (Fig. 3). The R_F of peak activity was 0.50 for the ash solution and 0.55 for the maple solution. In the maple solution, a second area of lesser activity was detected at R_F 0.33, corresponding to a slight peak in activity for the reference C^{14}-picloram. However, it is believed that the smaller peaks in both instances are artifacts caused by salts of the nutrient solution moving with picloram along the paper. When C^{14}-picloram was spotted alone (i.e., not spotted on top of the nutrient solution sample) and developed in the same manner, a single, relatively tight spot resulted at an R_F of about 0.50.

In the bioassay test, the R_F of high growth inhibition (Fig. 3) corresponded closely to the C^{14} activity peaks of the ash nutrient solution and the reference C^{14}-picloram spotted both with ash and maple solutions. However, the R_F of maximum inhibition for the maple nutrient solution did not correspond as closely to the peak of C^{14} activity.

Throughout the 22-day treatment period, observations were made on plant responses to the herbicide treatment. Six days after application of the herbicide, several trees showed slight leaf epinasty of the upper two or three whorls. By 22 days, most of the treated plants showed at least slight epinasty of the upper leaves, and several exhibited severe epinasty of the top whorl of leaves. Apical elongation also appeared to be retarded in several plants. However, no clear relationship of species and/or herbicide treatment with damage was apparent. The means of stem length, root length, and number of leaf whorls at 22 days are shown in Table 1.

TABLE 1. CONDITION OF ASH AND MAPLE TREES 22 DAYS AFTER APPLICATION OF C^{14}-PICLORAM AND C^{14}-2,4,5-T[a/]

Herbicide	Species	Length, cm		Number of Leaf Whorls
		Stem	Root	
Picloram	Ash	50.4±5.0	12.7±0.4	13.1±1.0
	Maple	42.4±2.4	13.7±0.7	13.7±1.0
2,4,5-T	Ash	50.7±2.8	14.3±1.1	12.3±0.8
	Maple	39.2±2.1	13.2±1.3	12.2±1.0

a. Application consisted of 24 µg of herbicide per plant. Each value is the mean ± the standard error of the mean.

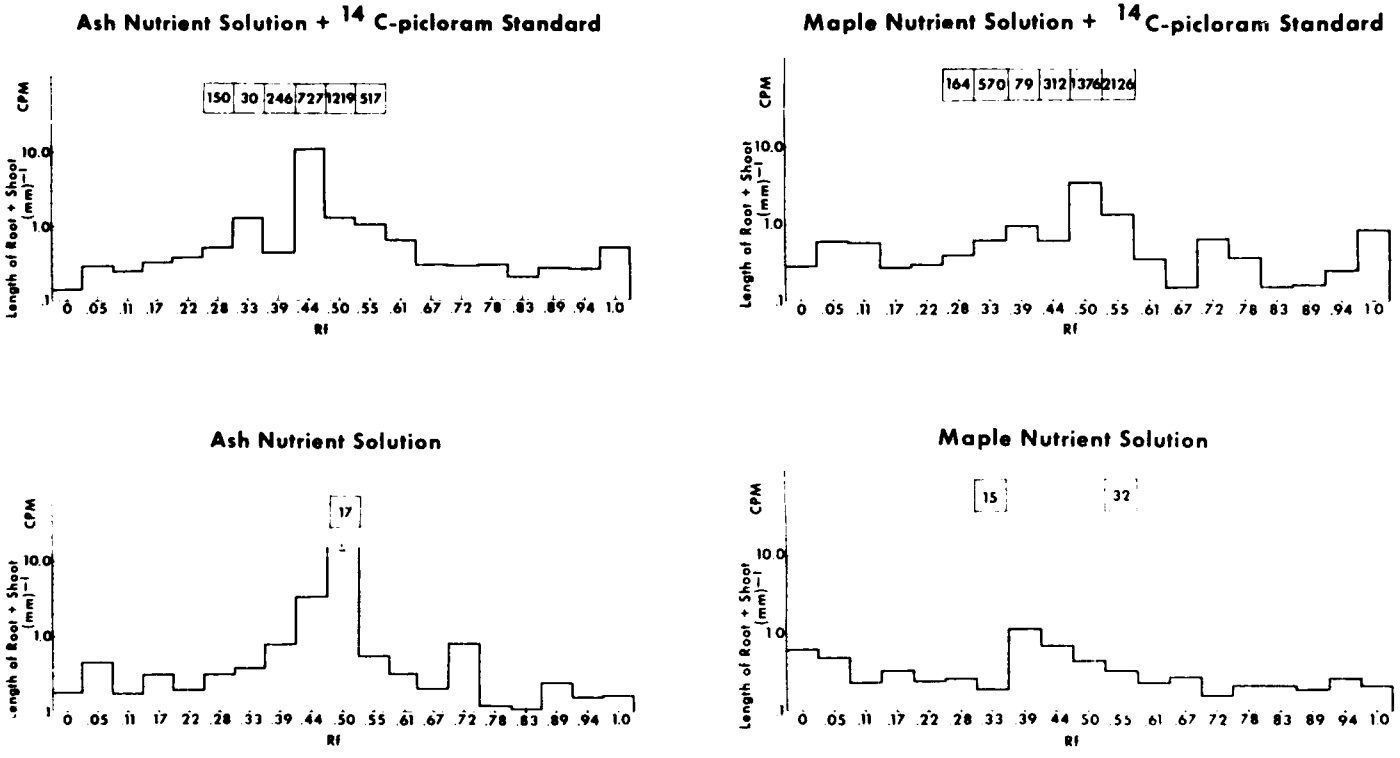

FIGURE 3. C^{14} Activity and Growth Inhibition Areas on Paper Chromatograms of Nutrient Solution of Ash and Maple Trees Treated with C^{14}-Picloram and of C^{14}-Picloram Standard Solutions Co-Chromatographed with the Nutrient Solutions. Histograms represent growth inhibition of germinated lettuce seed as a reciprocal of length of root and shoot in millimeters. Squares represent the C^{14} activity significantly above background at the 95% confidence level. Values are cpm corrected for background and counting efficiency.

At the end of the experiment, the range of pH for the nutrient solution of all plants was 7.0 to 7.3, an increase from an original pH of 6.1.

Table 2 presents the total quantities of C^{14} recovered in the leaf wash, in the treated leaves, and in the nutrient solution after 22 days. The balance of C^{14} in the plant was obtained by subtracting the recovered amounts from the amount originally applied to the leaves. At the 90% confidence level, significantly greater quantities of C^{14}-2,4,5-T were retained on the treated leaf surfaces than C^{14}-picloram, as indicated by the leaf wash values. However, there was no significant herbicide or species difference in the quantity retained in the treated leaves. The loss of C^{14} to the nutrient solution by the ash treated with picloram was significantly lower than the other treatments. The significant difference between the balance of C^{14}-picloram and C^{14}-2,4,5-T in the plant is primarily a reflection of the amount recovered in the leaf wash.

The autoradiographs of the excised treated leaves show little evidence of any smearing of C^{14} during the rinsing procedure (Fig. 4). Images on the film show the localized areas where the labeled herbicides were applied. There appears to be little difference in the C^{14} distribution in the leaves or petioles.

TABLE 2. RECOVERY AND LOCATION OF C^{14}-LABELED PICLORAM AND 2,4,5-T[a/]

Herbicide	Species	Recovered C^{14}, cpm[b/]			Unrecovered Balance in Plant, cpm
		Leaf Wash	Leaf Content	Nutrient Solution	
Picloram	Ash	97,733[a] ±13,805	15,298[a] ±5,549	1,790[a] ±371	107,180[a] ±11,199
	Maple	109,161[a] ±23,235	15,409[a] ±8,384	6,298[b] ±3,446	91,133[a] ±10,116
2,4,5-T	Ash	150,330[b] ±14,288	11,199[a] ±2,192	2,403[ab] ±665	58,068[b] ±12,797
	Maple	163,125[b] ±24,803	13,191[a] ±5,073	4,298[ab] ±3,433	41,386[b] ±19,786

a. Application consisted of 24 µg of herbicide per plant (220,000 dpm).

b. Values are means ± standard error of the mean times the t value for 90% confidence limits. Means followed by the same superscript letter are not significantly different.

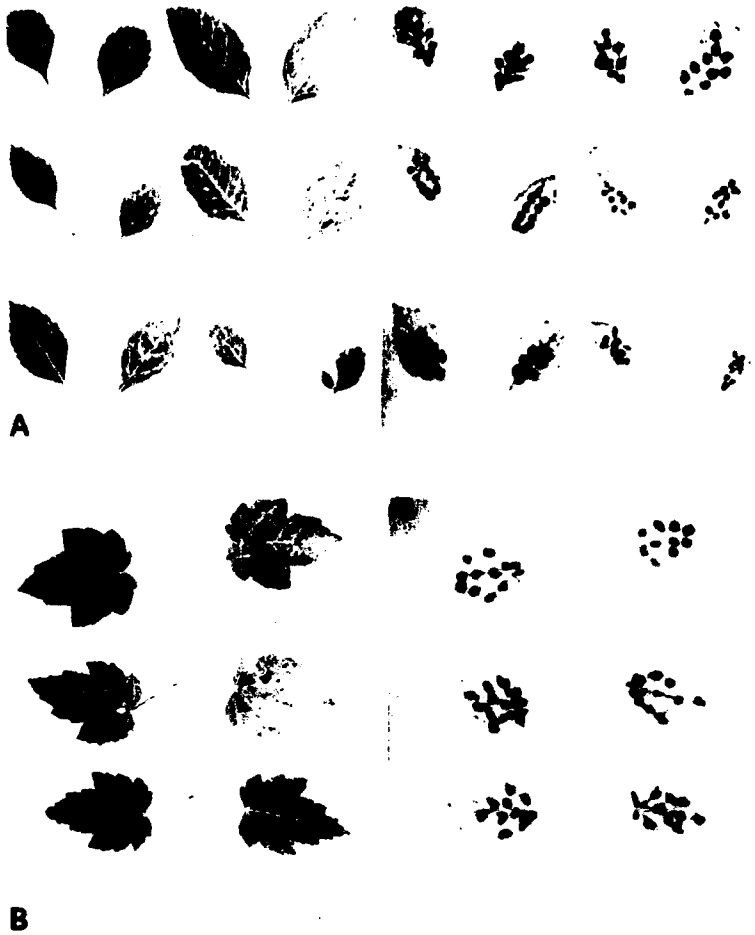

FIGURE 4. Autoradiographs of Leaf Pairs Treated with C^{14}-Labeled Herbicides. (A) Leaves of green ash, each treated with 0.05 μc (12 μg) of C^{14}-picloram in 95% ethanol. (B) Leaves of red maple, each treated with 0.05 μc (12 μg) of C^{14}-2,4,5-T in 95% ethanol.

The autoradiographs of the whole plants appear anomalous when compared with the calculated balance of activity expected in the whole plant (Table 2). Images produced on film were much more evident for the upper foliage of the plants treated with C^{14}-2,4,5-T than for those treated with C^{14}-picloram, yet the calculated balance indicated the opposite. Figure 5 shows representative ash and maple seedlings treated with C^{14}-2,4,5-T. Based on a subjective rating of film image density for different parts of the plant, root and lower stem sections produced images of approximately equal density for plants treated with both C^{14}-2,4,5-T and C^{14}-picloram. However, the images produced by the upper foliage parts of the C^{14}-picloram plants were considerably less than those for C^{14}-2,4,5-T. Expression of C^{14} on a specific activity basis of stem, leaf, and root samples removed from two trees of each treatment and counted in liquid scintillation substantiated the C^{14} distribution indicated by the autoradiographs (Fig. 5).

This discrepancy may be related to an unaccounted loss of C^{14} from the picloram-treated plants. Considering that the C^{14}-picloram balance in the plant was calculated to be about twice as great as the balance of C^{14}-2,4,5-T, it seems logical to expect that a loss could have occurred at the treated leaf surface or in the rinse procedure. However, if there had been an incomplete rinse of the leaf surface it would have been reflected in a larger quantity in leaf content. It has been reported that photodegradation of picloram can occur[17], and it is possible that a considerable amount of C^{14} was lost from the leaf surface by this means over the 22-day period. We did, however, apply quantities of the same two labeled herbicides on glass discs under the same environmental conditions used for the trees. After 48 hours, under 1,750 ft-c (8.7×10^4 ergs per cm^2 per sec) at the disc surface on a 16-hour photoperiod at 26 C, no significant loss of C^{14} was detected for either C^{14}-picloram or C^{14}-2,4,5-T. Once the herbicide entered the plant, C^{14} could have been lost through the evolution of $C^{14}O_2$, although there is no evidence in the literature that indicates that picloram is readily metabolized in the plant. In contrast, Meikle, Williams, and Redemann[18] reported that very little $C^{14}O_2$ was lost from cotton plants alone over a 15-day period after treatment with carboxyl-labeled C^{14}-picloram. They did find a much higher level of $C^{14}O_2$, however, when the evolution was measured from soil containing cotton plants. Meikle et al.[18] concluded that the cotton plant did not contribute significantly to decomposition of picloram.

Considering that the indicated amount of picloram in the plants may not be completely reliable for the above reasons, Figure 6 represents the percentage distribution of C^{14}-picloram and C^{14}-2,4,5-T after 22 days. Although the data appear to indicate that the percentage of herbicide lost from the roots is relatively small, it becomes quite significant when considered as a percentage of the herbicide that actually entered the plant from the treated leaves. For example, 1.9% lost to the nutrient solution from maple treated with 2,4,5-T, when converted to a percentage of the quantity that actually entered the plant from the treated leaves, becomes 9.4%.

FIGURE 5. Autoradiographs of Representative Plants, (A) Green Ash and (B) Red Maple, Treated Foliarly with 0.1 μc (24 μg) C^{14}-2,4,5-T. Treated leaves were removed prior to autoradiography. Numerical values represent specific activities of tissue samples removed at indicated locations after autoradiography. Values are cpm/g dry weight after correction of count rates for background and efficiency. NS signifies that the activity of the particular sample was not significant above background at the 95% confidence level.

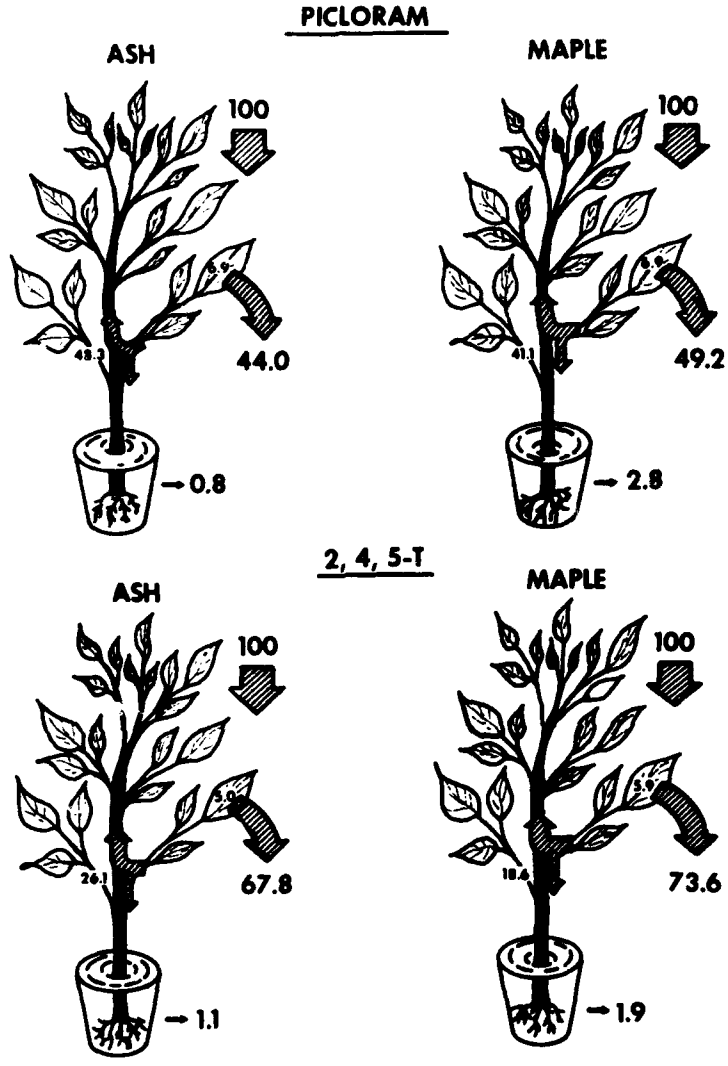

FIGURE 6. Diagrammatic Representation of the Distribution and Loss of C^{14}-Picloram and C^{14}-2,4,5-T When Applied to Green Ash and Red Maple Leaves. Values (as percentage) show the C^{14} activity applied (0.1 μc); amounts recovered in leaf wash, treated leaves and nutrient solution; and the unrecovered balance in the whole plant.

Microautoradiography of the ash and maple stem and petiole tissues failed to produce a clear picture of the distribution of either C^{14}-2,4,5-T or C^{14}-picloram at the cellular level. Preservation of the tissues was good and cellular detail was clear. Resolution of the silver grains against background was also adequate. In both ash and maple treated with C^{14}-picloram, there appeared to be no apparent strong localization in any tissues except for occasional concentration in xylem vessels. Localization was much more apparent in the trees treated with C^{14}-2,4,5-T. Stem sections of ash above the site of application showed silver grains concentrated on the xylem vessel walls and in the vascular ray tissue (Fig. 7), while sections below the site of application showed silver grains along the vessel walls of the xylem but without a sharp localization. In the petiole sections of the treated ash leaves, breakdown and disruption of phloem parenchyma was evident (Fig. 8). Some localization of silver grains in the phloem fibers was found, but silver grains were also predominant in the xylem vessels.

In sections removed from maple treated with C^{14}-2,4,5-T, silver grain distribution was somewhat similar to that of ash except that localization in vascular ray tissue was not observed in the upper stem sections.

It appears that both herbicides utilize phloem and xylem in moving throughout the plant. In many tissue sections examined, both ash and maple showed disruption of phloem tissues as well as the presence of C^{14}. Localization of silver grains on the walls of the lumina of the xylem vessels was noted in all tissues of ash and maple. The heavy concentration of C^{14} along the vascular ray tissue in ash may indicate a pathway for movement of C^{14}-2,4,5-T between xylem and phloem.

B. WHITE ASH TREATED WITH C^{14}-PICLORAM

Figure 9 shows the cumulative total C^{14} activity of the nutrient solution of each replicate (two trees per pot) over the period of 9 days. The variability in the quantity of C^{14} lost to the nutrient solution by each pair of trees is quite evident. It is interesting to note that the maxima and minima of the activity of the various replicates generally occur at similar times. The loss of 19,200 cpm at day 9 for one of the replicates represents 4.3% of the total C^{14}-picloram applied.

Two days after the C^{14}-picloram was applied to the white ash, slight to moderate epinasty of the upper two whorls of leaves was evident. By day 8, all treated trees had severe epinasty of the upper two whorls of leaves and terminal growth had ceased in the majority of plants.

The autoradiographs of the white ash trees showed a greater quantity of C^{14} distributed throughout the plant compared with the C^{14}-picloram-treated green ash and red maple. This difference is thought to be attributed to the presence of 0.2% Tween 20 in which the C^{14}-picloram was applied rather than a species difference. We have found that a given quantity

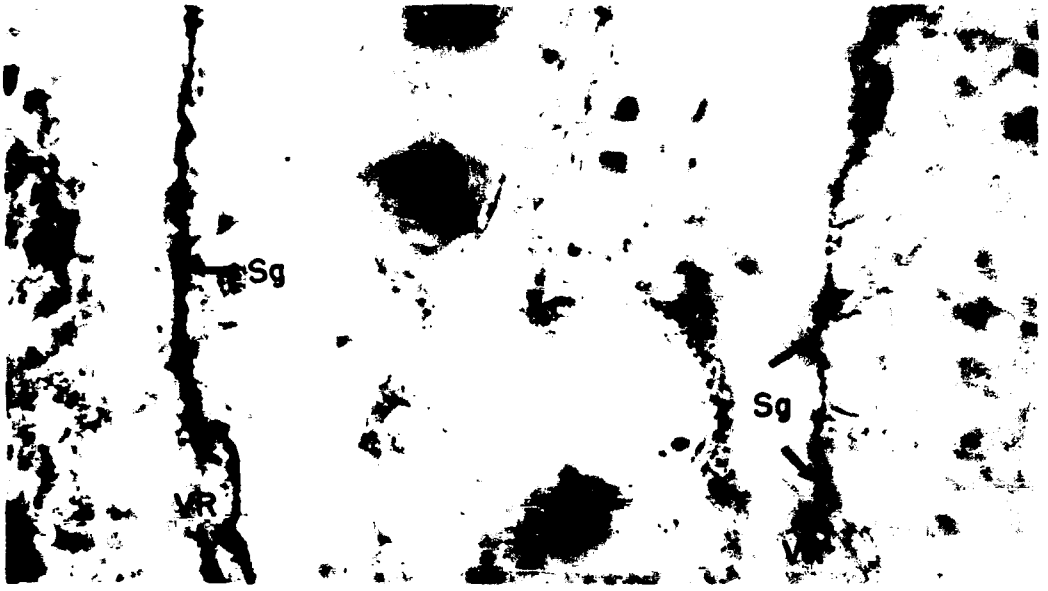

FIGURE 7. Microautoradiograph of a Cross-Section of Xylem Stem Tissue Above the C^{14}-2,4,5-T-Treated Leaves of Green Ash. C^{14} is strongly localized in vascular ray elements. sg, silver grains; VR, vascular ray; xv, xylem vessel.

FIGURE 8. Microautoradiograph of a Cross-Section Removed from the Petiole of a C^{14}-2,4,5-T-Treated Leaf of Green Ash. C^{14} is distributed in phloem and xylem. C, cortex; CA, cambium; P, phloem; X, xylem; pf, phloem fibers; sg, silver grains; xv, xylem vessel.

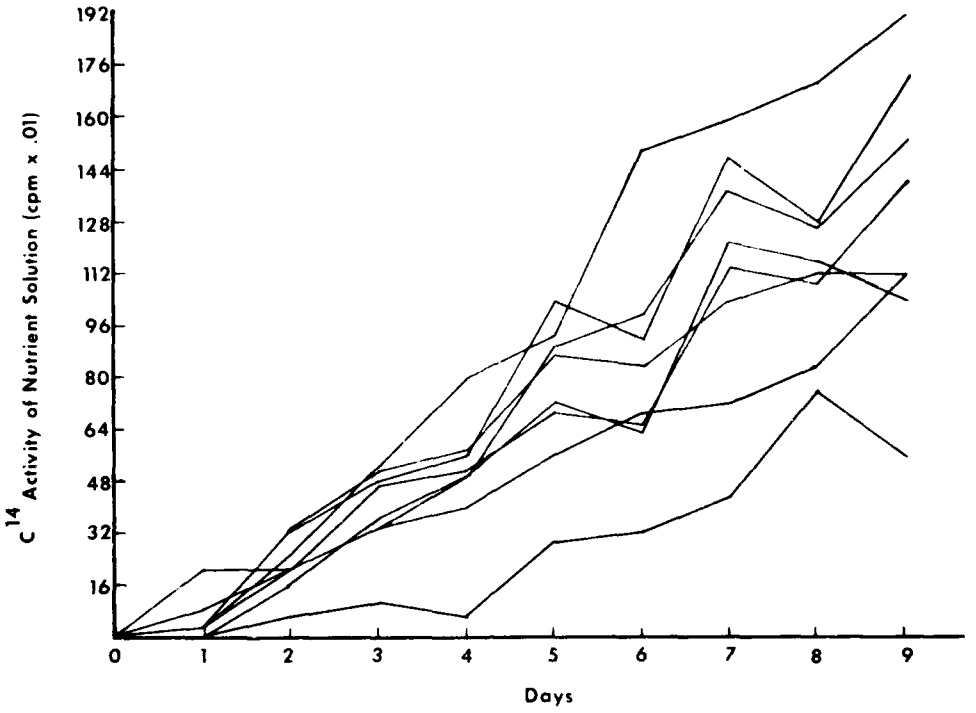

FIGURE 9. Cumulative C^{14} Loss from Roots of White Ash After Foliar Treatment with 0.1 μc (24 μg) of C^{14}-Picloram. Each plotted line represents one replicate consisting of two trees in 800 ml of nutrient solution.

herbicide applied in an aqueous solution with Tween 20 will move into the plant better than the same quantity applied in 95% ethanol. This difference is probably explained in part by the slower evaporation of the Tween 20 solution from the leaf surface and a resulting longer period for movement into the leaf. Similar results were found by J. W. Brown* using alcoholic and Tween 20 solutions on bean plants.

IV. CONCLUSIONS

The loss of C^{14}-picloram and C^{14}-2,4,5-T from the roots of red maple, green ash, and white ash was demonstrated. Chromatography of the nutrient solutions suggested that the C^{14} activity detected was associated with the unaltered herbicide molecules. The quantities of herbicides lost from the roots appeared to have no consistent relationship with species tolerance or resistance. This suggests that root exudation, as a detoxification mechanism, does not determine species tolerance to these particular herbicides. This would be in agreement with the results of the investigations of Linder, Mitchell, and Freeman[19], who found no relationship between the potency of a compound as a growth regulator and the ability of a plant to exude the compound.

The amounts of C^{14}-picloram and C^{14}-2,4,5-T lost from the roots in the investigations reported here could be of considerable importance. The ecological significance of root-excreted substances having effects on other organisms (allelopathy) has been recognized by many investigators (e.g., Mitchell and Linder[10], Mitchell, Smale, and Preston[20], van Overbeek[21] and Woods[22]). Although Mitchell and Linder[10] believed that root exudation of exogenous compounds is of no practical significance, they did acknowledge that some exuded substances may persist in the soil and affect nearby plants or the growth of subsequent crops. At the time of publication of the above-mentioned article, Mitchell and Linder stated that regulating substances exuded from roots were confined to two families of compounds: alpha-methoxyphenylacetic acids and chlorinated benzoic acids. Since that time, the exudation of 2,4-D[6,15] and picloram[5,23], both exceptions to the above classification, have been shown. The exudation of picloram and 2,4,5-T reported in the present investigations would not fall within this classification. The exudation of picloram may be of greater ecological importance than the exudation of previously reported compounds because of its reported persistence in soils[24,25].

* Unpublished data.

A strong comparison of results between trees treated with 2,4,5-T and those treated with picloram cannot be confidently made because of the unexplainable discrepancy in the distribution of the latter herbicide. However, valid conclusions can be drawn within each herbicide treatment, i.e., a comparison of species response.

The autoradiographs indicate that 2,4,5-T was distributed throughout ash and maple in greater amounts than picloram, although both moved to the lower stem and root system. The acropetal and basipetal translocation of C^{14}-2,4,5-T after movement out of the leaves of ash and maple is in marked contrast to the results of Leonard, Bayer, and Glenn[4], who found that almost no 2,4,5-T moved out of the treated leaves of red maple and only slight movement occurred out of white ash leaves. This lack of transport was thought to be related to phloem injury. Pallas[3] found good distribution of C^{14}-2,4,5-T in red maple but poor translocation in white ash. He reported that 76% of the applied 2,4,5-T was unabsorbed after 16 days and 6% was found within the disc excised from the treated areas of the leaf. These data agree rather closely with our findings at 22 days. Pallas[3] believed that poor absorption of 2,4,5-T by leaves of white ash may account for its resistance. However, our results with green ash indicate that a greater quantity of 2,4,5-T was transported out of the treated leaves than with maple. It must be realized, of course, that it is difficult to make direct comparisons between various reported investigations concerning the application of 2,4,5-T and picloram to ash and maple because of different environmental conditions, age of plant material, method of herbicide application, and other known and unknown variables.

The possibility that detectable amounts of exogenous compounds may be lost to the rhizosphere from roots should be considered in order to attain a better understanding of the physiological and possible ecological aspects of using growth-regulating compounds for woody plant control.

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13 ABSTRACT

Liquid scintillation and autoradiography were utilized in conjunction with paper chromatography to study the uptake, distribution, and root exudation of foliarly applied C^{14}-picloram (4-amino-3,5,6-trichloropicolinic acid) and C^{14}-2,4,5-T (2,4,5-trichlorophenoxyacetic acid) in red maple, green ash, and white ash.

A leaf-washing technique, which allowed direct counting of the herbicide retained on the surface of the treated leaves, revealed that a greater amount of 2,4,5-T than of picloram was washed off the leaves of all species after 22 days. Greater penetration of picloram was noted when the herbicide was applied with 0.2% Tween 20 as opposed to application with 95% ethanol.

Autoradiography indicated that 2,4,5-T was distributed throughout ash and maple tissues in greater quantities than picloram, and microautoradiography of green ash stem tissue showed possible xylem to phloem exchange. Both picloram and 2,4,5-T were translocated acropetally and basipetally in all species, indicating utilization of both the apoplast and symplast by these two herbicides.

Significant root loss of both picloram and 2,4,5-T was demonstrated in all three species. Greater root loss of both herbicides occurred in red maple than in the two species of ash.

Bioassay in conjunction with paper chromatography and liquid scintillation counting suggested that the C^{14} activity in the (over)

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exudates was associated with the unaltered herbicide molecules for both 2,4,5-T and picloram. The quantities of 2,4,5-T and picloram exuded from the roots appeared to have little relationship to herbicide tolerance or resistance of these species.

14. Key Words

Absorption
Root exudation
2,4,5-Trichlorophenoxyacetic acid
Picloram
Herbicides
Translocation
Ash
Maple